

We claim:

1. An isolated mammalian c-kit-/c-met- cardiomyocyte precursor cell of muscular origin.
2. The cell of claim 1, wherein the cell is a human cell.
3. The cell of claim 1, wherein the cell is a mouse cell.
4. The cell of claim 1, wherein the cell is from a fetus, a child, or an adult.
5. The cell of claim 1, wherein the cell is in suspension.
6. The cell of claim 1, wherein the cell is between about 3  $\mu\text{m}$  and 10  $\mu\text{m}$  in diameter.
7. The cell of claim 6, wherein the cell is approximately 4  $\mu\text{m}$  in diameter.
8. The cell of claim 1, wherein the cell differentiates into a cardiomyocyte.
9. The cell of claim 1, wherein the cell differentiates into a spontaneously beating cardiomyocyte.
10. The cell of claim 1, wherein the cell is transduced with a viral vector.

FOOT-00420001

Sub  
A1

11. The cell of claim 1 wherein the viral vector comprises a heterologous nucleic acid.
12. The cardiomyocyte of claim 8, wherein the cardiomyocyte expresses GATA-4, troponin-T, L-type calcium channel, or Nkx2.5, or a combination thereof.
13. A method of isolating a c-kit-/c-met- cardiomyocyte precursor cell of muscular origin, comprising:
  - separating cells of less than 40  $\mu\text{m}$  in diameter from a suspension of muscle cells;
  - culturing the cells in a tissue culture medium on a solid substrate; and
  - isolating the cells in suspension in the medium; thereby isolating the c-kit-/c-met- cardiomyocyte precursor cell of muscular origin.
14. The method of claim 13, wherein separating cells of less than 40  $\mu\text{m}$  in diameter from a suspension of cells comprises:
  - passing the suspension of cells through a first filter with a pore size of about 50 – 200  $\mu\text{m}$  to collect a first eluate containing cells of greater than about 50  $\mu\text{m}$  and less than about 200  $\mu\text{m}$  in diameter; and
  - passing the first eluate through a second filter with a pore size of about 40  $\mu\text{m}$  to collect a second eluate containing cells of less than about 40  $\mu\text{m}$  in diameter.
15. The method of claim 14 wherein the first filter has a pore size of at least 100  $\mu\text{m}$  and the second filter has a pore size of about 40  $\mu\text{m}$ .

Sub A

16. The method of claim 13, wherein the tissue culture medium is a growth medium.
17. The method of claim 16, wherein the growth medium is supplemented with a growth factor.
18. The method of claim 17, wherein the growth factor is EGF, or bFGF, or a combination thereof.
19. The method of claim 18, wherein the growth factor EGF is present at a concentration between about 5 and 50 ng/ml.
20. The method of claim 19, wherein the growth factor EGF is present at a concentration between about 5 and 10 ng/ml.
21. The method of claim 19, wherein the growth factor EGF is present at a concentration of about 10 ng/ml.
22. The method of claim 18, wherein the growth factor bFGF is present at a concentration between about 5 and 50 ng/ml.
23. The method of claim 22, wherein the growth factor bFGF is present at a concentration between about 5 and 10 ng/ml.
24. The method of claim 22, wherein the growth factor bFGF is present at a concentration of about 10 ng/ml.
25. A mammalian c-kit-/c-met- cardiomyocyte precursor cell of muscular origin isolated according to the method of claim 13.

TOP SECRET

SUB  
A1

26. A method for differentiating a c-kit-/c-met- cardiomyocyte precursor cell of muscular origin, comprising:
- separating cells of less than 40  $\mu\text{m}$  in diameter from a suspension of muscle cells;
  - culturing the cells in a tissue culture medium in the presence of a growth factor on a solid substrate;
  - isolating the cells in suspension in the medium; and
  - removing the growth factor, thereby differentiating the c-kit-/c-met- cardiomyocyte precursor cell of muscular origin into a cardiomyocyte.
27. The method of claim 26, wherein the cardiomyocyte is spontaneously beating.
28. The method of claim 26, wherein the growth factor is EGF, or bFGF, or a combination thereof.
29. The method of claim 28, wherein the growth factor EGF is present at a concentration between about 5 and 50 ng/ml.
30. The method of claim 29, wherein the growth factor EGF is present at a concentration between about 5 and 10 ng/ml.
31. The method of claim 29, wherein the growth factor EGF is present at a concentration of about 10 ng/ml.
32. The method of claim 28, wherein the growth factor bFGF is present at a concentration between about 5 and 50 ng/ml.
33. The method of claim 32, wherein the growth factor bFGF is present.

F02207-004E0001

Sub  
A1

at a concentration between about 5 and 10 ng/ml.

34. The method of claim 32, wherein the growth factor bFGF is present at a concentration of about 10 ng/ml.
35. A mammalian cardiomyocyte differentiated from a c-kit-/c-met-cardiomyocyte precursor cell of muscular origin according to the method of claim 26.
36. A method of treating a myocardial injury in a subject, comprising administering a therapeutically effective amount of the cell of claim 1, thereby treating the myocardial injury.
37. The method of claim 36, wherein the cells are introduced locally into the myocardial injury.
38. The method of claim 36, wherein the cells are introduced systemically into the subject.
39. The method of claim 38, wherein the cells are introduced intravenously.
40. The method of claim 36, wherein the myocardial injury is cardiomyopathy, myocardial infarction or congenital heart disease.
41. A method of treating cardiac muscle dysfunction, comprising administering to a subject with cardiac dysfunction a therapeutically effective amount of mammalian c-kit-/c-met- cardiomyocyte precursor cells of muscular origin that differentiate into beating cardiomyocytes.

FOOTNOTES

SUB  
A1

42. The method of claim 41, wherein the cardiac muscle dysfunction is a myocardial infarction, a cardiomyopathy, or a congenital heart disease.
43. A pharmaceutical composition comprising mammalian c-kit-/c-met-cardiomyocyte precursor cells of muscular origin in a pharmaceutically acceptable carrier.
44. A method for screening for an agent to determine the effect of the agent on a cardiomyocyte comprising:
- providing mammalian c-kit-/c-met- cardiomyocyte precursor cells of muscular origin;
  - contacting the cells with the agent; and
  - observing the effect of the agent on the cells.
45. The method of claim 44, wherein observing the effect comprises determining the effect of the agent on differentiation of the cells.
46. The method of claim 45 wherein determination of the effect on differentiation comprises assaying expression of GATA-4, expression of cardiac troponin-T, expression of L-type calcium channel, or expression of Nkx2.5, or a combination thereof.
47. The method of claim 45, wherein observing the effect comprises assaying a parameter of cardiomyocyte function of the cells.
48. The method of claim 47 wherein the parameter comprises spontaneous beating of the cells.
49. A kit for promoting cardiomyocyte differentiation, comprising a container containing a purified population of mammalian c-kit-/c-

102201-001E0001

SUB  
AI

